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Pharmacognostic and Phytochemical Analysis of *Asystasia Variabilis* Trim. - An Extrapharmacopoeal Ayurvedic Medicinal Plant

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ABSTRACT

Background - *Asystasia Variabilis* Trim. also known as *Maithaala Kaddi* by traditional practitioners of Udupi. Belonging to the family *Acanthaceae*. Is a semi-scandent herb, and effectively used by the Folklore practitioners for the management of *Amlapitta* (acid peptic disorder), worm infestations and rheumatism. **Aim -** To explore the pharmacognostical and preliminary phytochemical parameters of plant to standardize the drug. **Materials and Methods -** whole mature plant of *A.variabilis* was collected from Udupi district. Macroscopic, microscopic physico-chemical standards, HPTLC and secondary metabolites screening were scientifically recorded. **Results -** The pharmacognostical leaf study has shown single layered epidermis throughout midrib and lamina. Anatomical features of stem exhibit single layer of epidermis covered with trichomes. Inner to this, densely arranged collenchyma cells are present. In transverse section of root showed epidermal layer is surrounded by root hairs which are abundant. Powder microscopy characteristics showed the presence of starch in parenchyma region, mesophyll cells with stomata and sclerides were present. The standard out print of the drug is represented by physicochemical standards and HPTLC. Preliminary Phytochemical study shows that it contains Alkaloids, Carbohydrates, Steroids, Tannins and Phenol. **Conclusion -** Pharmacognostical study carried out on *A.variabilis* showed quality standards of the drug, with respect to its macroscopy, microscopy, physico-chemical standards and HPTLC.

Key words: *Asystasia variabilis, Macro-Microscopic, Physico-chemical standards.*

INTRODUCTION

Ayurveda opines there is no plant which has no medicinal value and which cannot be utilized as medicine.[1] Many such golden treasures still lie today in the dense green which are yet to be discovered.

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Also this knowledge is followed only by a few groups of folklore practitioners. In present era the demand for herbal medicine is increasing day by day, as more than 70% of population in developing countries is following traditional system of medicine for primary health care.

On the other hand we can say India is a rich treasure trove of diverse flora along with ethno-medicinal knowledge. Ethno medicine is the study or comparison of the traditional medicine practiced by various ethnic groups.[2] Traditional knowledge encompasses wisdom, knowledge, teaching and experience of these communities and many a times it is orally transmitted from generation to generation.[3] Among such drug least *Asystasia variabilis* Trim. which is known as *Maithaala Kaddi* in Udupi dist. This belongs to *Acanthaceae* family and is a semi-scandent

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herb. Its stem is acutely quadrangular. This plant generously grows during rainy season throughout Udupi dist.[4] And these are effectively used by the Folklore practitioners for the management of *Amlapitta* (acid peptic disorder), rheumatism and worm infections. Traditional method involves squeezing the whole plant in the water and used to grind the rice and internally in the form of different dishes. Therapeutically the same water is used to treat *Pitta Vikaras* by adding a little quantity of jaggery.[5]

Detailed Pharmacognostical studies of this plant have not reported till now, hence the present study was undertaken, in order to fix some standards for its identification and can use in clinical practice.

Materials and Methods

Collection of Sample

The fresh plant sample of *Asystasia variabilis* Trim. were collected in the month of April 2017 from Udupi Dist. Karnataka, India. A voucher specimen of the leaf is authenticated and deposited in Dept. of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

Macroscopy

By following the standard procedures the morphological characters of leaves, stem and roots were visually observed and studied. Using Canon IXUS digital camera the external features of the test samples were documented using size indicating rulers.[6] Authentication of the macroscopic features was done comparing to local flora.

Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.[7]

Powder microscopy

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerin. Slides were observed under the microscope, diagnostic characters marked and photographed with Zeiss Axio Cam camera under bright field light.

Physico-chemical parameters

As per the Ayurvedic Pharmacopoeia of India *A.variabilis* whole plant powder was tested for pharmacopoeial constants like loss on drying, percentage of total ash, water-soluble ash, acid-insoluble ash, was calculated.[8]

Preliminary phyto-chemical analysis

For the preliminary phytochemical analysis, 5 g. powdered drug was extracted, dried and weighed. The presence or absence of various phytoconstituents viz. Alkaloids, Steroids, Carbohydrates, Tannins, Saponins, Terpenoid, Coumarins, Phenols, Carboxylic acid, Amino acids, Resin, Quinine and Flavonoids were detected by usual prescribed methods.[9]

HPTLC finger printing

1g. of *Asystasia variabilis* (whole plant) powder was extracted with 10 ml of *alcohol*. 3, 6 and 9µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm. using Linomat 5 TLC applicator. The plate was developed in Toluene : Ethyl acetate (7.0 : 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. R_f, color of the spots and densitometric scan were recorded.[10],[11]

Observations and Results

Macroscopic evaluation

Belongs to *Acanthaceae* family is a semi-scandent herb, stem acutely quadrangular. Leaves variable, up to 12x5 cm, elliptic, ovate or narrowly lanceolate,

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acuminate at apex, base narrowed, glabrous; petiole up to 2cm long. Racemes terminal, lax, 7-13 cm long. Calyx–lobes linear-lanceolate corolla up to 1.8 cm long, pale pinkish –violet dotted red. Capsule up to 2.5 cm long, narrowly clavate, seeds ovate–deltoid to orbicular. (Figure 1)

Figure 1: Macroscopy of *A.variabilis* Trim.

Microscopic evaluation

T.S. of Leaf

T.S. of leaf divided into 3 parts, lamina, midrib and vascular bundle region. Epidermis is single layered throughout midrib and lamina, on the lower side lower epidermis continuous single layered. Covering type of trichomes is present on both the upper and lower epidermis. Beneath the epidermis in the midrib region there are 3-4 layers of compactly arranged cells of collenchyma. Lamina is divided into 2 portions- palisade layer and spongy parenchyma. Palisade layer- single layered, radially elongated cells present. Spongy parenchyma is with intercellular space, ground tissue is mostly parenchymatous collateral conjoint open type of vascular bundle present, pericyclic fibre is present on outer side of vascular bundle which is very thin.

T.S. of Midrib contains layer of epidermis which is continuous in both upper and lower there is 2-3 layer of collenchyma cells below upper epidermis and above lower epidermis. Ground tissue is mostly parenchymatous. Arch shaped vascular bundle present in central region also 2 small vascular bundle present in midrib region. Trichomes are present in upper and lower epidermis which are covering type. (Figure 2a, 2b, 2c, 2d)

Figure 2: Transverse Section of leaf of *A.variabilis*

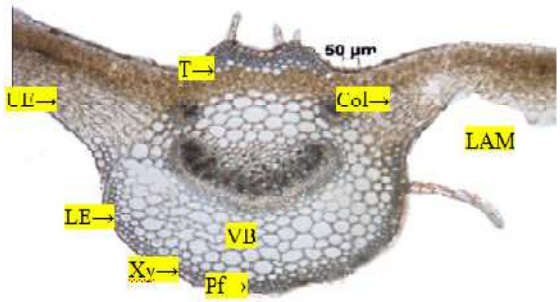

Fig2a: T.S of leaf

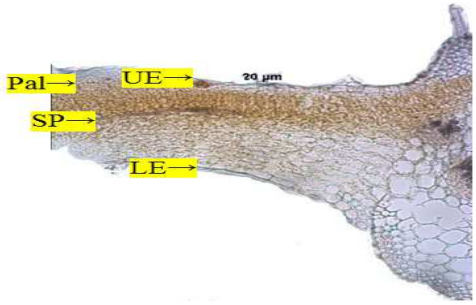

Fig 2b: T.S of lamina

UE – upper epidermis; LAM – lamina; LE – lower epidermis; Pal – palisade; PF – pericyclic fibres; Ph – phloem; SP – spongy parenchyma; T–trichomes; VB–vascular bundle; Xy – xylem

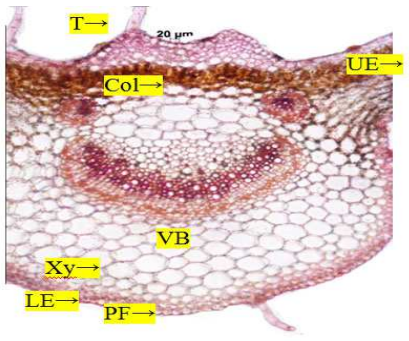

Fig 2c: T.S of midrib of *A.variabilis*

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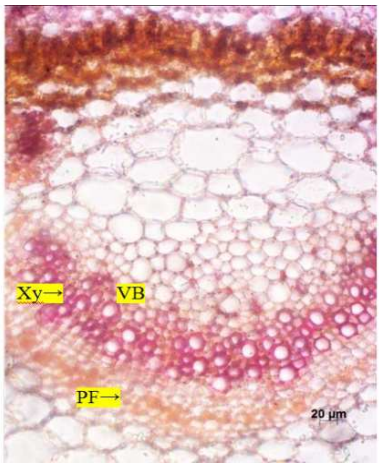

Fig 2d: T.S of midrib enlarged

UE – upper epidermis; LE – lower epidermis Pal – palisade; PF – pericyclic fibres; Ph – phloem; T–trichomes; VB–vascular bundle; Xy – xylem.

T.S. of Stem

T.S. of stem showed rectangle in outline with ridges. Beneath the epidermis there is compactly arranged collenchyma cells. Cortex parenchymatous cells thick walled. Phloem cells are on outerside. Xylem cells are on inner side. Xylem consists of vessels and xylem rays. Pith cells are parenchymatous thick walled polygonal cells. (Figure 3a,3b)

Figure 3: Microscopy of stem of *Asystasia variabilis*

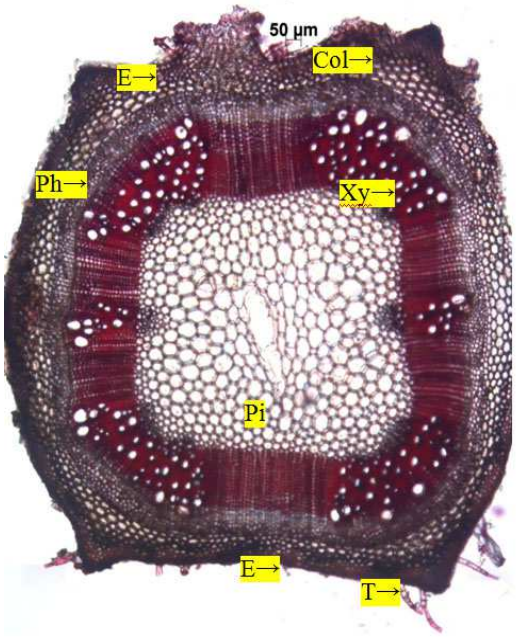

Fig 3a: TS of stem

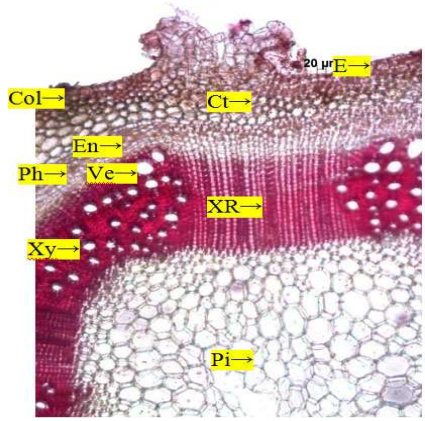

Fig 3b: A portion of stem enlarged

Col – collenchyma; Ct – cortex; E – epidermis;En - endodermis; Ph – phloem; Pi – pith Ve – vessel ; Xy – xylem; Col – collenchyma; Ct – cortex; Pa – parenchyma; XR – xylem rays.

T.S. of Root

In transverse section root showed epidermal layer is surrounded by root hairs which are abundant. Epidermis is single layered followed by cortex which is wide 3-4 layers parenchymatous, which is followed by endodermis inner to this there is vascular bundles. (Figure 4)

Figure 4: Microscopy of root of *Asystasia variabilis*

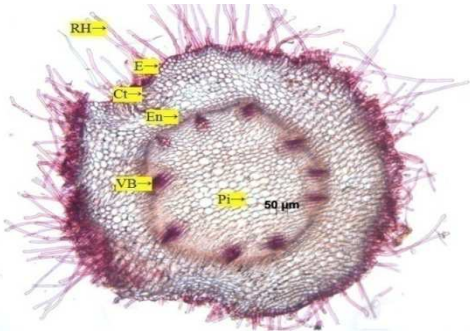

Fig 4a: TS of root

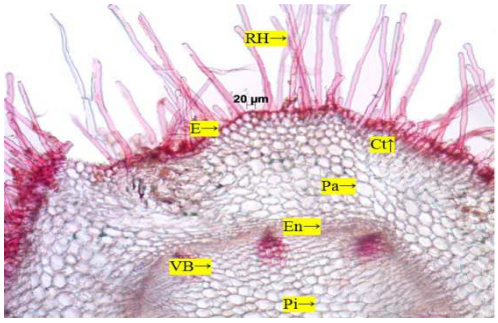

Fig 4b: A portion enlarged

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Ct – cortex; . E-epidermis; En - endodermis;Pa – parenchyma; Pi – pith; RH – root hairs; VB – vascular bundle.

Powder microscopy

Powder showed the presence of starch in parenchyma region, mesophyll cells with stomata, collapsed trichomes and vessels with reticulate thickening, collapsed vessel, bundle of fibres and sclerides. (Figure 5)

Figure 5: Powder microscopy of leaf and petiole of *Asystasia variabilis*

Fig 5a: Parenchyma with starch

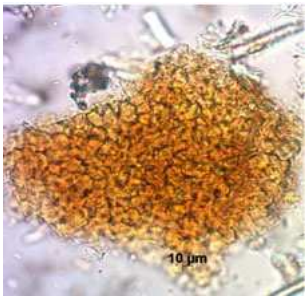

Fig 5b: Mesophyll cells with stomata

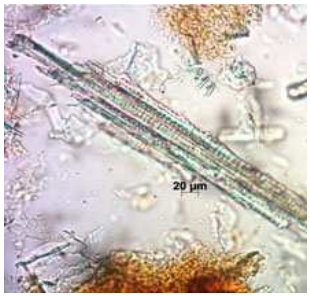

Fig 5c: Bundle of fibres

Fig 5d: Collapsed vessels

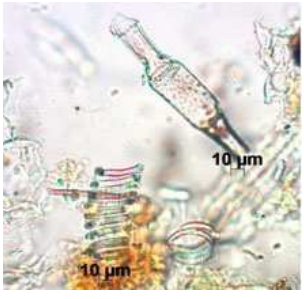

Fig 5e: Collapsed trichomes and vessels

Fig 5f: Sclerides with reticulate thickening

Physico-chemical analysis

Physico-chemical standards were a representative of its purity, physical nature and chemical trait. *A.variabilis* whole plant powder was tested for loss on drying at 105°c total ash, acid insoluble ash, ethanol and water soluble extractive as per standard protocol. Results of standardization parameters of *Asystasia variabilis* Trim are estimated and represented in (Table 1.)

Table 1: Results of standardization parameters of *Asystasia variabilis* Trim.

Parameter	Results n = 3 %w/w
Loss on drying	12.90
Total Ash	10.68
Acid Insoluble Ash	0.2
Water soluble Ash	3.3
Alcohol soluble extractive value	3.42
Water soluble extractive value	22.7

Phytochemical study

Preliminary phytochemical test were conducted for *A.variabilis,* test for alkaloids (Dragendroff's test, Wagner's test, Mayer's test and Hager's test), carbohydrates (Moloch's test, Fehling's test and Benedict's), steroids (Libermann-Burchard and Salkowski), saponins, phenols, coumarin, triterpenoids, quinine, resin and tannins were conducted and result displayed (Table 2). The preliminary phytochemical studies are essential to know the chemical constituents present in the drug. Action of any drug depends upon active principles present in it.

Table 2: Results of preliminary phytochemical screening of *Asystasia variabilis* Trim.

Test	Inference
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	+

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Flavonoids	-
Saponins	-
Terpenoid	-
Coumarins	-
Phenols	+
Carboxylic acid	-
Amino acids	-
Resin	-
Quinone	-

Tests	Colour if positive	Alcoholic extract
Alkaloids		
Dragendroff's test	Orange red precipitate	Orange red precipitate
Wagner's test	Reddish brown precipitate	Reddish brown precipitate
Mayer's test	Dull white precipitate	Dull white precipitate
Hager's test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann-buchard test	Bluish green colour	Bluish green colour
Salkowski test	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer
Carbohydrate		
Molish test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Dark green
Flavonoids		
Shinoda's test	Red or pink	Grey colour
Saponins		
With $NaHCO_3$	Stable froth	No froth
Triterpenoids		
Tin and thionyl chloride test	Pink/Red colour	Grey colour
Coumarins		
With 2 N NaOH	Yellow	Green colour
Phenols		
With alcoholic ferric chloride	Blue to blue black or brown colour	Blue black colour
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
Amino acid		
With ninhydrin reagent	Purple colour	Green colour solution
Resin		
With aqueous acetone	Turbidity	No turbidity
Quinone		
Conc. sulphuric acid	Pink/purple/red	Green colour solution

HPTLC

HPTLC finger print profile of ethanol extract of *A.variabilis* has been obtained with suitable solvent system. The developed plates were visualized under UV light and white and then under light after derivation with vanillin sulphuric acid reagent. Rf, color of the spots and densitometric scan at 254 and 366 nm were recorded. On photo documentation there were 6 bands under short UV, 9 bands under long UV and 7 bands after derivatisation with vanillin sulphuric acid reagent (Table 3, Figure 6). Densitometric scan at 254nm showed 9 peak in which at Rf of 0.01 (20.54%), 0.53 (18.17%), 0.78 (24.03%), showed maximum peak (Figure 6a). Densitometric scan at 366nm showed 9 peak in which at Rf of 0.53

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(27.585%), Rf 0.78 (29.91%) showed maximum peak (Figure 6b) where as at 620nm there were 8 peak in which at Rf of 0.44 (34.61%) showed maximum peak. (Figure 6c).

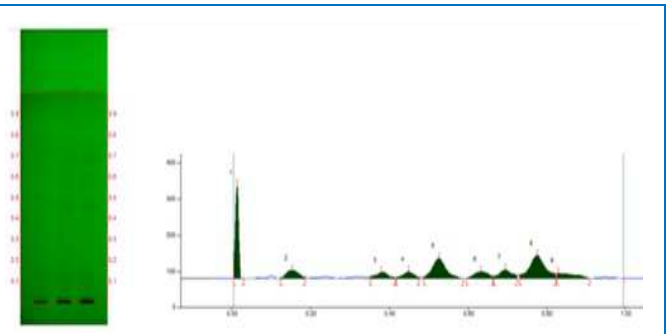
Table 3: R_f values of samples

Short UV	Long UV	After derivatisation
0.13 (L. green)	0.13 (F. red)	0.13 (L. purple)
-	0.18 (F. red)	0.18 (D. purple)
-	-	0.37 (D. purple)
0.39 (L. green)	0.39 (F. red)	-
0.45 (D. green)	0.45 (F. red)	0.45 (L. purple)
0.54 (L. green)	0.54 (F. red)	0.54 (L. purple)
0.61 (L. green)	0.61 (F. red)	0.61 (L. purple)
0.68 (D. green)	0.68 (F. red)	0.68 (D. purple)
-	0.77 (F. red)	-
-	0.90 (F. red)	-

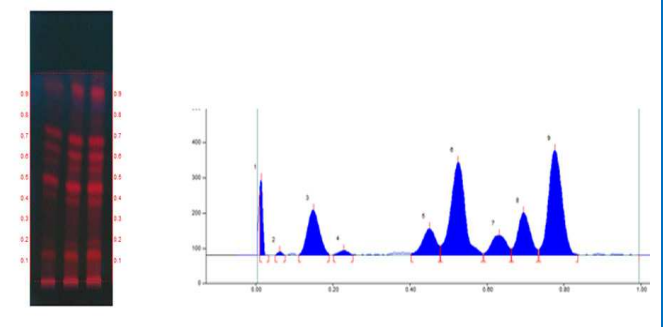
Solvent system – Toluene: Ethyl Acetate (7:1)

*F – Fluorescent; L –Light; D – Dark

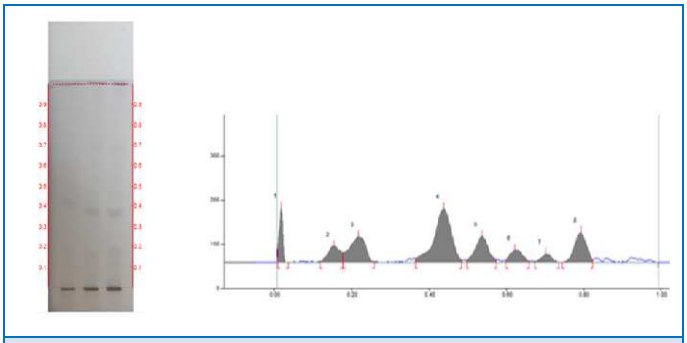
Figure 6: HPTLC of ethanolic extract of whole plant of *A.variabilis* Trim.



6a: Under short UV **6.b: Densiometric scan at 254 nm**



6c: Under long UV **6d: Densiometric scan at 366nm**



6e: Under white light **6f: Densiometric scan at 620nm (after derivatisation)**

DISCUSSION

The macroscopic features observed can be further used for preliminary identification of the particular plant. Microscopic recordings proved to be beneficial in establishing the authenticity and detection of substitutes/adulteration for herbal raw drugs. *A.variabilis* is a semi-scandent herb. Its stem is acutely quadrangular. The microscopic findings of leaf have shown single layered epidermis throughout midrib and lamina. Anatomical features of stem of *A.variabilis* shown single layer of epidermis covered with trichomes which are covering type. Inner to this, densely arranged collenchyma cells are present. In transverse section of root showed epidermal layer is surrounded by root hairs which are abundant. Epidermis is single layered followed by cortex which is wide 3-4 layers parenchymatous. Powder microscopy characteristics showed the presence of starch in parenchyma region, mesophyll cells with stomata and sclerides were present. The total inorganic composition of the drug indicated by the total ash was found to be 10.68 w/w. The silicacious matter recorded by acid insoluble ash was found to be 0.2 w/w. The quantity of ash which is easily soluble in water indicated by water soluble ash which is 3.3% w/w. Moisture and volatile matter content in sample represented by loss on drying is found to be 12.90% w/w. The approximate amount of the course powder's chemical constituents is indicated by the extract obtained by filtering it. Water soluble extractive value of the test sample obtained was 22.7w/w and alcohol soluble was 3.42w/w. All these

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pharmacopoeial parameter helps to assess the quality and purity of herbal drugs.

A preliminary phytochemical test was conducted using the water extracts showed the presence of Steroids, Carbohydrate, alkaloids, Tannin and Phenols. This preliminary analysis of chemical composition helps in studying the detail chemistry of herbs. The presence of phyto-constituents with different Rf values was revealed in HPTLC photo documentation. Densitometric scan of the plates showed diagnostic bands under 254 nm, 366 nm and post derivatisation. HPTLC finger printing is an effective and latest procedure of screening herbal raw drugs for its standardization.

CONCLUSION

Knowing the traditionally used and commonly available plants will be an extra benefit to the present knowledge. *Asystasia variabilis* Trim. is a member of *Acanthaceae* family is a less known medicinal drug, but used by traditional practitioners in worm infestation, rheumatism and *Amlapitta* (acid peptic disorders). The results of the pharmacognostical, physico-chemical analysis and HPTLC profiling helps in standardization with respect to its purity, identity genuinity of herbal material. Preliminary phytochemical screening showed the presence of Alkaloids, Carbohydrates, Steroids, Tannins, and Phenol. Conducting researches in a systematic way are the best method for the application of the folk medicine. Hence the pharmacognostical study is one of the major criteria for identification as well as to evaluate the therapeutic benefit of plants. Further clinical research study can be carried out in the management of certain related diseases.

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